STIC-ILL

Adam's only $20.00

From: Schultz, James
Sent: Wednesday, July 09, 2003 8:39 PM
To: STIC-ILL
Subject: lit request 09/695,451

Hello,
Could you please obtain the following:

BRAIN RESEARCH, (1997 May 30) 758 (1-2) 99-106.

J. Douglas Schultz, Ph.D.
AU 1635 (Biotechnology)
Patent Examiner
United States Patent and Trademark Office
(703) 308-9355
(703) 746-3973 (fax)
Office: CM1 12E18
Mail: CM1 11E12

Article title	Distribution of tumor necrosis factor receptor messenger RNA in normal and herpes simplex virus infected trigeminal ganglia in the mouse
Article identifier	0006899397026468
Authors	Cunningham_E_T_Jr Stalder_A_K Sanna_P_P Liu_S_S Bloom_F_E Howes_E_L_Jr Campbell_I_L Margolis_T_P
Journal title	Brain Research
ISSN	0006-8993
Publisher	Elsevier Netherlands
Year of publication	1997
Volume	758
Issue	1-2
Supplement	0
Page range	99-106
Number of pages	8
User name	Adonis
Cost centre	Development
PCC	$20.00
Date and time	Thursday, July 10, 2003 8:31:00 AM

ELSEVIER

Brain Research 758 (1997) 99–106

BRAIN RESEARCH

Research report

Distribution of tumor necrosis factor receptor messenger RNA in normal and herpes simplex virus infected trigeminal ganglia in the mouse

Emmett T. Cunningham Jr. [a,b], Anna K. Stalder [c], Pietro Paolo Sanna [c], Stephanie S. Liu [a], Floyd E. Bloom [c], Edward L. Howes Jr. [b], Iain L. Campbell [c], Todd P. Margolis [a,b,*]

[a] *The Francis I. Proctor Foundation, UCSF, Medical Center (Box 0944), San Francisco, CA 94122-0944, USA*
[b] *The Department of Ophthalmology, University of California, Medical Center, San Francisco, CA 94122, USA*
[c] *Department of Neuropharmacology, Scripps Research Institute, La Jolla, CA 92037, USA*

Accepted 22 January 1997

Abstract

Purpose: to investigate the distribution of p55 and p75 tumor necrosis factor (TNF) receptor mRNA in normal murine trigeminal ganglia, and in murine trigeminal ganglia acutely infected with McKrae strain herpes simplex virus (HSV). *Methods:* in situ hybridization with antisense ^{35}S-labeled riboprobes for mRNA encoding both the p55 and p75 TNF receptor (TNFR) subtypes was used in normal and HSV-infected murine trigeminal ganglia. Sense riboprobes were used as controls. *Results:* in situ hybridization with both p55 and p75 riboprobes produced a strong autoradiographic signal over many, but not all, trigeminal sensory neurons. Signal for mRNA encoding both TNFR subtypes was also present over the arachnoid layers surrounding trigeminal ganglia. Acute ocular HSV infection was accompanied by an intense leukocytic infiltrate into the ophthalmic portion of the trigeminal ganglia, and, in this setting, increased p55 and p75 mRNA signal was closely related to the location and number of infiltrating white blood cells. The distribution and number of trigeminal sensory neurons expressing mRNA for the two TNFR subtypes did not appear to change following infection. Signal over control sections hybridized with sense p55 and p75 TNFR cRNA probes was comparable to background. *Conclusions:* the observed distribution of p55 and p75 TNFR mRNA over trigeminal sensory neurons and over the arachnoid layers surrounding trigeminal ganglia supports suggestions that TNF has a direct effect on neurons, either as a neuromodulator or neurotrophic factor, and that TNF may play a central role in blood–brain barrier regulation. Increased signal for TNFR mRNA in acutely infected trigeminal ganglia appears to reflect infiltration by receptor-bearing white blood cells.

Keywords: Cytokine; Trigeminal ganglion; Meninges; Receptor; Tumor necrosis factor (TNF)

1. Introduction

Ocular inoculation with herpes simplex virus (HSV) results in both productive and latent pathways of viral gene expression in trigeminal sensory neurons [5,23,27,29,33,34,52]. Determinants of HSV latency appear complex, and include both host and viral factors [18,48]. Much recent attention has been given to the role of the host immune system and cytokines in viral latency and reactivation [1,19,39], particularly tumor necrosis factor (TNF), a potent pro-inflammatory and mitogenic signalling molecule [9,57].

The role of TNF in HSV infection has been controversial. Rossol-Voth and colleagues have shown that systemically administered TNF protects against HSV-induced mortality in vivo [42], and both Feduchi and associates [17] and Chen and colleagues [47] have described inhibition of HSV replication by TNF in cell culture. Of note, the in vitro study by Chen and associates demonstrated TNF-induced inhibition of HSV replication in both corneal fibroblasts and corneal epithelial cells [47], findings highlighted by the report of Ghiasi and associates correlating local TNF expression with decreased HSV-induced corneal scarring in mice [21]. In contrast, Waley and associates have recently reported enhanced HSV reactivation and replication in explanted trigeminal ganglia superfused with TNF [59]. Taken together, these findings suggest that the effect of TNF on HSV replication may be site and tissue dependent, determined presumably by cell-specific expression of TNF receptor (TNFR) subtypes.

* Corresponding author. Fax: +1 (415) 476 0527.

PII S0006-8993(97)00169-8

Two high affinity TNFR subtypes have been identified to date, termed p55 and p75 [9,24,57]. While trigeminal sensory neurons appear to be an important site of HSV latency, and TNF does appear to influence HSV reactivation in trigeminal ganglia [59], evidence has yet to be provided for synthesis of TNFRs by trigeminal ganglion neurons and/or neuroglial cells. We, therefore, applied a sensitive and specific in situ hybridization protocol [24] to examine the distribution of mRNA encoding the two TNFR subtypes in normal murine trigeminal ganglia, and in murine trigeminal ganglia acutely infected with HSV.

2. Materials and methods

Studies in this report were carried out in accordance with the Use of Laboratory Animals as adopted by the National Institutes of Health. All experiments were performed on 8- to 10-week-old female BALB/c mice (Simonsen, Gilroy, CA).

2.1. Ocular HSV infection

Mice were anesthetized with CO_2. A few drops of 0.5% proparacaine (Alcon, Fort Worth, TX) were then applied to the corneas, the epithelium was lightly scored with a sterile 27-gauge syringe, and 20–30 μl of McKrae strain HSV (10^9 PFU/ml) were applied topically. The mice were allowed to recover from anesthesia and maintained for 3 days following inoculation.

2.2. Tissue preparation

Deeply anesthetized normal and infected mice were perfused transcardially with 10 ml of 0.15 M NaCl, followed immediately by 20 ml of 10% neutral buffered formalin. The trigeminal ganglia were removed and allowed to postfix in the final perfusate at 4°C for 2–3 weeks, after which time they were transferred to the same perfusate with 10% sucrose added as a cryoprotectant for 12–24 h. Four 1-in-4 series of 10 μm-thick cryostat sections through the entire trigeminal ganglia were collected onto uncoated, positively charged slides (Fisher).

2.3. In situ hybridization histochemistry

Bluescript SK transcription vectors (Stratagene) containing the full-length cDNA for the p55 and p75 murine TNF receptors were generously provided by Dr. Raymond Goodwin of Immunex Corporation [21]. Detailed protocols for probe synthesis, prehybridization, hybridization, posthybridization, and autoradiographic localization have been published previously [14]. In brief, high specific activity sense and antisense riboprobes were synthesized by incorporation of both ^{35}S-labelled CTP and UTP with specific activities greater than 800 Ci/mmole with the Riboprobe II System (Promega). Full-length templates used to generate antisense probes were obtained by linearization of the p55 and p75 cDNAs with Bgl II and Eco RI, respectively, and by use of T3 RNA polymerase for transcription. Full-length p55 and p75 sense probes used as controls were linearized with BamH I and Sac I, respectively, and transcribed with T7 RNA polymerase. Mounted sections were further postfixed in 10% neutral buffered formalin for 30 min, after which they were treated with proteinase K, acetylated, and dehydrated. The 3^{35}S-labeled cRNA probes were diluted in hybridization buffer at 10^7 cpm/ml, applied to the sections, and allowed to hybridize at 55–60°C for 12–24 h. Sections were then treated with RNase A to reduce background, and washed through progressively lower concentrations of saline-sodium citrate to reduce the salt content and increase the stringency of hybridization. Finally, sections were dehydrated, exposed to Beta Max film (Amersham) for 4–7 days, dipped in Kodak nuclear emulsion NTB3, dried, exposed for 2–4 weeks, developed, and counterstained with hematoxylin–eosin.

3. Results

3.1. Uninfected trigeminal ganglia

A total of 12 trigeminal ganglia from 8 animals were hybridized with antisense probes for mRNA encoding p55 and p75 TNFR subtypes. In each case, signal was observed over many, but not all, trigeminal sensory neurons in all portions of the ganglia, and diffusely over the arachnoid layers surrounding the trigeminal ganglia (Figs. 1–3). Signal for p55 TNFR mRNA was qualitatively denser than that for p75 TNFR mRNA in every case. While signal over some neuroglial cells could not be excluded with certainty, particularly over those cells in close approximation to intensely labeled trigeminal sensory neurons, signal over neuroglial cells in axon-rich portions of the trigeminal ganglia was comparable to background with both p55 and p75 riboprobes.

3.2. HSV-infected trigeminal ganglia

A total of eight trigeminal ganglia from four animals acutely infected with McKrae strain HSV 3 days previously were hybridized with antisense probes for mRNA encoding the p55 and p75 TNFR subtypes. Ganglia were taken from those animals previously shown to have an active and intense acute ocular infection [24]. In each case, signal for mRNA encoding both p55 and p75 was observed over trigeminal sensory neurons in all portions of the ganglia, and over the arachnoid layers surrounding the ganglia, with no qualitative difference in the distribution or intensity of mRNA signal as compared to uninfected trigeminal ganglia. However, ocular HSV infection was

accompanied by a marked leukocytic infiltrate into the medial, or ophthalmic, portion of the trigeminal ganglia, and an intense autoradiographic signal for both p55 and p75 was observed over infiltrating white blood cells in this setting (Figs. 1 and 2). Again, while label over those neuroglial cells in close approximation to intensely labeled

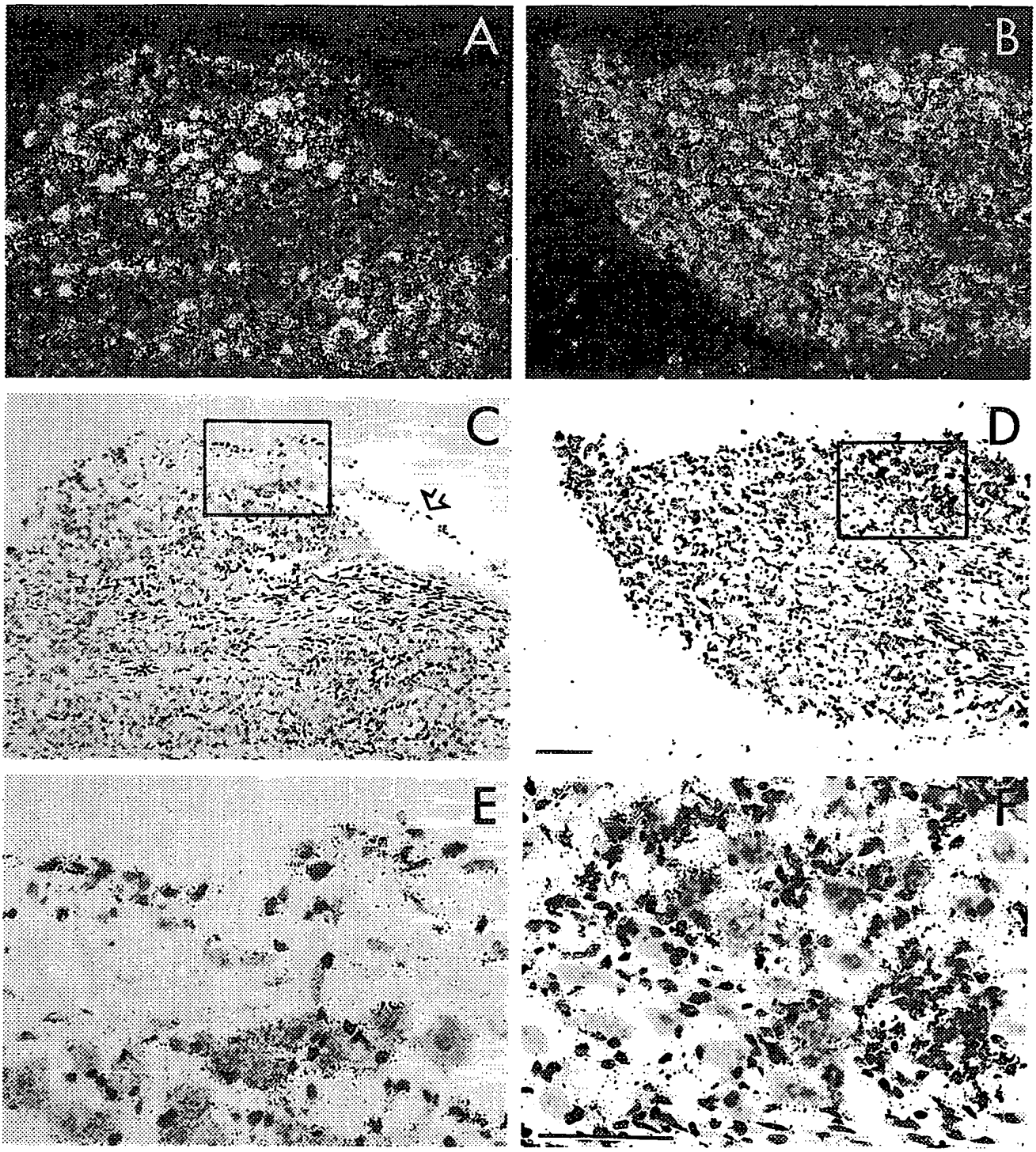

Fig. 1. Darkfield (A,B) and brightfield (C–F) photomicrographs of sections through normal (left panel) and HSV-infected (right panel) trigeminal ganglia demonstrating the in situ distribution of autoradiographic signal for mRNA encoding the p55 TNF receptor. Intense signal was observed over many, but not all, sensory neurons in all portions of both normal and HSV-infected ganglia (E,F). Although not addressed quantitatively, signal intensity appeared not to increase over trigeminal sensory neurons following infection (F). Heavy signal was also present over the surrounding arachnoid layers in both normal and HSV-infected ganglia (C, open arrow), and over infiltrating white blood cells in the medial portion of the trigeminal ganglia following ocular infection (right panel, see F). Axon-rich portions of the sections are labeled (*). Magnified photomicrographs (E,F) are taken from boxed areas of low-power brightfield images (C,D). Sections were counter-stained with hematoxylin–eosin. Scale bars at 200 μm.

trigeminal sensory neurons could not be absolutely excluded, the signal over neuroglial cells in axon-rich portions of the ganglia was comparable to background for both p55 and p75.

3.3. Controls

A total of five uninfected and four infected trigeminal ganglia were hybridized with sense probes for mRNA

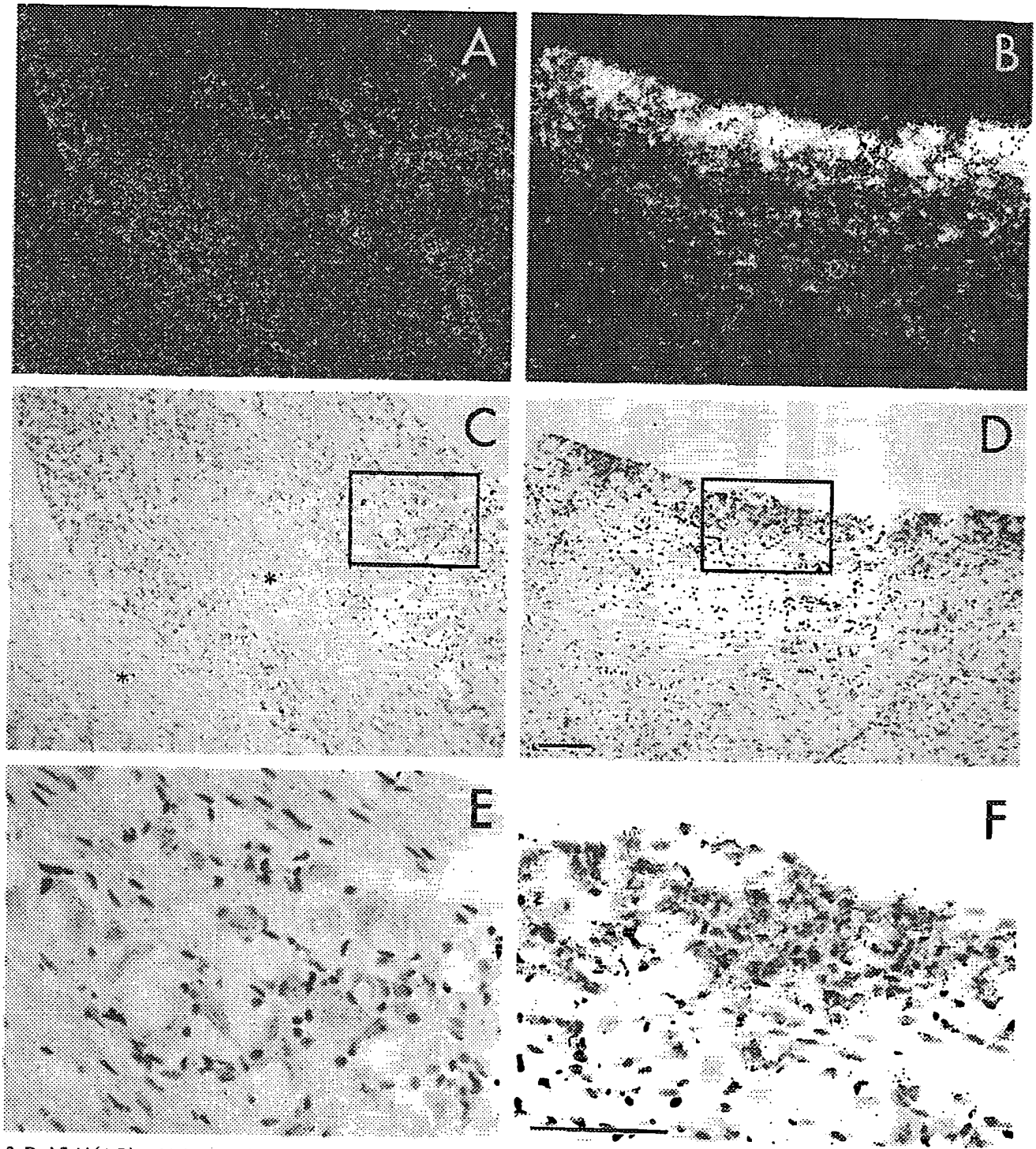

Fig. 2. Darkfield (A,B) and brightfield (C–F) photomicrographs of sections through normal (left panel) and HSV-infected (right panel) trigeminal ganglia demonstrating the in situ distribution of autoradiographic signal for mRNA encoding the p75 TNF receptor. Light signal was observed over many, but not all, sensory neurons in all portions of both normal and HSV-infected ganglia (E,F). Signal intensity appeared not to increase over trigeminal sensory neurons following infection, although intense signal was observed over infiltrating white blood cells in the medial, or ophthalmic, portion of the trigeminal ganglia in this setting (right panel, see F). Light signal was also present over the surrounding arachnoid layers in both normal and HSV-infected ganglia (not shown). Axon-rich portions of the sections are labeled (*). Magnified photomicrographs (E,F) are taken from boxed areas of low-power brightfield images (C,D). Sections were counter-stained with hematoxylin–eosin. Scale bars at 200 μm.

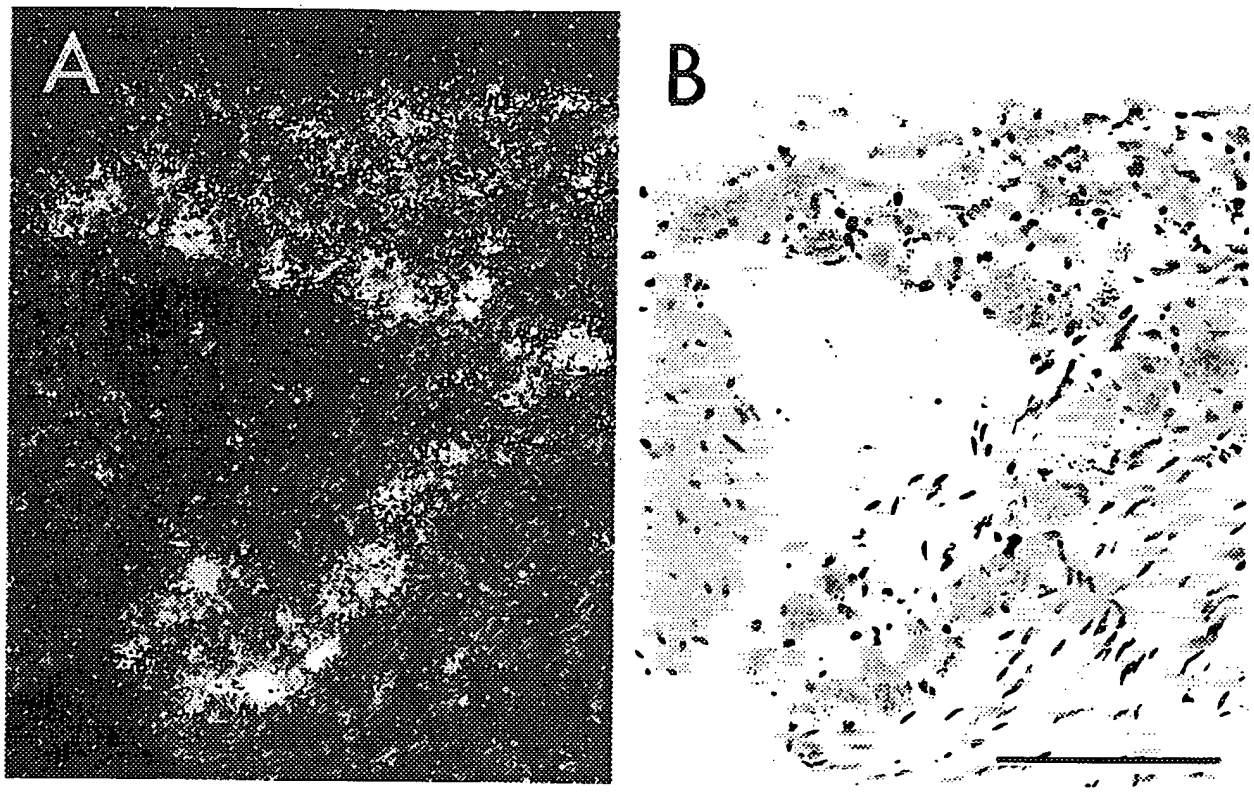

Fig. 3. High-power darkfield (A) and brightfield (B) photomicrographs of the same section through a normal trigeminal ganglion to show the in situ distribution of autoradiographic signal for mRNA encoding the p55 TNF receptor. Heavy signal is present over many, but not all neurons. Sections were counter-stained with hematoxylin–eosin. Scale bar at 200 μm.

encoding the p55 and p75 the TNFR subtypes. In each case, autoradiographic signal over both the trigeminal ganglia and their surrounding arachnoid layers was comparable to background (Fig. 4). In addition, we recently used a sensitive and specific RNA protection assay [54] to demonstrate mRNA encoding both TNFR subtypes in normal

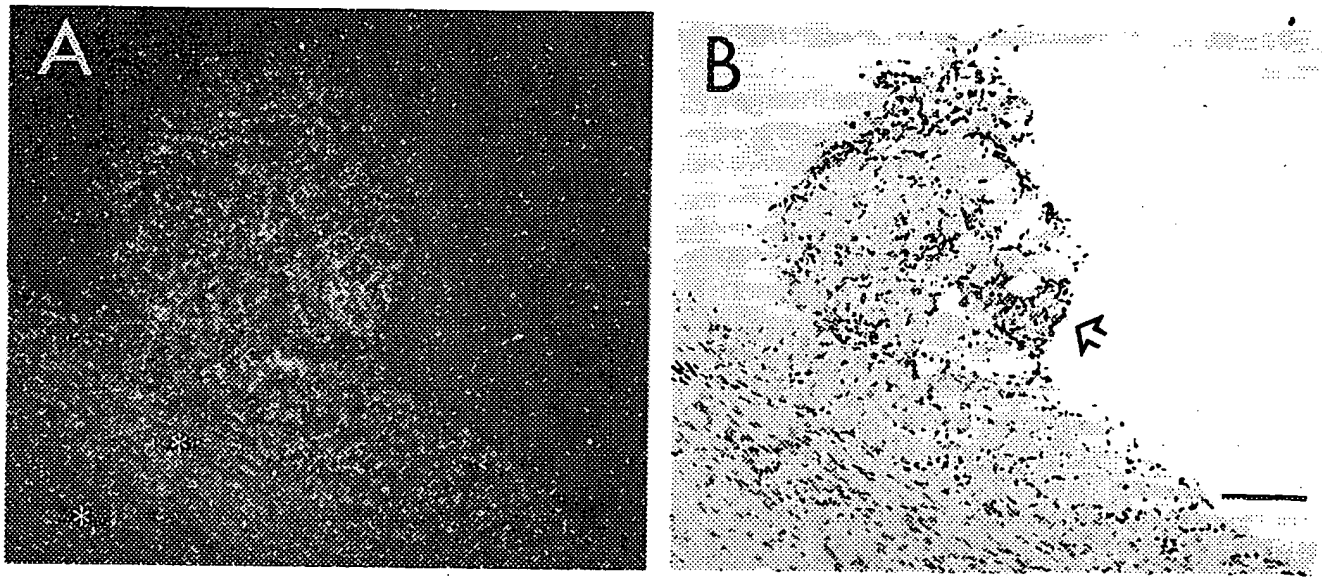

Fig. 4. Control darkfield (A) and brightfield (B) photomicrographs of the same section through the medial, or ophthalmic, portion of an HSV-infected trigeminal ganglion demonstrating the in situ distribution of autoradiographic signal using a sense probe for mRNA encoding the p55 TNF receptor. Signal using this, as well as the sense riboprobe for the p75 TNF receptor, was comparable to background. Axon-rich portions of the section are labeled (*). Sections were counter-stained with hematoxylin–eosin. Scale bar at 200 μm.

murine trigeminal ganglia (unpublished observation), supporting our in situ results. Lastly, these TNFR probes have been used in both uninfected and HSV infected mouse eye [15] and lymphocytic choriomeningitis virus (LCMV)-infected brain [53,54] with high sensitivity and specificity.

4. Discussion

In situ hybridization was used to localize mRNA encoding the p55 and p75 TNFR subtypes in normal murine trigeminal ganglia, and in murine trigeminal ganglia acutely infected with McKrae strain HSV. Signal for mRNA encoding both TNFR subtypes was present over many, but not all, trigeminal sensory neurons in all portions of the ganglia, and over the arachnoid layers surrounding the trigeminal ganglia. Acute ocular infection with McKrae strain HSV appeared not to qualitatively affect the distribution or intensity of p55 or p75 mRNA signal over trigeminal sensory neurons or cells in the arachnoid layers, but was accompanied by a marked white blood cell infiltrate into the medial, or ophthalmic, portion of the trigeminal ganglia. In this setting, infiltrating white blood cells expressed high levels of mRNA for both TNFR subtypes.

Autoradiographic signal could not be identified over isolated trigeminal neuroglial cells in either normal or HSV-infected animals, a finding most evident over axon-rich portions of the ganglia. However, due to the relatively high energy of ^{35}S-labeled riboprobes, the close approximation of stellate neuroglial cells to trigeminal sensory neurons, and the loss of cellular morphology following the heavy proteinase K treatment required by in situ hybridization protocols [14], the possibility that some neuroglial cells in the immediate vicinity of TNFR expressing trigeminal neurons, specifically satellite cells, were also labeled by p55 and/or p75 riboprobes, cannot be excluded with certainty.

While this study represents the first demonstration of TNFRs on neurons in the peripheral nervous system, considerable anatomical evidence supports the existence of TNFR production by neurons and/or neuroglial cells in the central nervous system. Kinouchi and associates were the first to suggest the presence of high affinity TNFR sites in mouse brain homogenates using ^{125}I-murine-TNF [28]. Stalder and Campbell confirmed and extended this finding using a sensitive RNase protection assay, thus identifying mRNA encoding both the p55 and p75 TNFR subtypes in whole mouse brain RNA preparations [54]. More recently, in vitro studies have demonstrated mRNA [25] and immunoreactivity [13] for the p55 TNFR subtype in rat hippocampal and cortical neurons, as well as high affinity binding of ^{125}I-murine-TNF to mouse astrocytes via a 58 kDa receptor, presumed to be p55 [3,6].

The cellular function of neuronal and neuroglial TNFRs appears complex, with several studies suggesting a role for TNF in the regulation of both neurotransmission and cell survival. In the central nervous system, TNF has been shown to alter synaptic activity in rat hippocampal slice [56] and culture [25], to regulate the release of norepinephrine from nerve terminals in the rat hippocampus [26] and median eminence [16], and, paradoxically, to both potentiate [12,20,55], and abrogate [18] the toxic effects of such stimuli as glucose deprivation and excitatory amino acid exposure. The effect of TNF on central neuroglial cells has been less well studied, although TNF has been shown to decrease potassium currents [50] and promote apoptosis [32,41] in oligodendrocytes. In astrocytes, TNF has been shown to increase C3 gene expression [7], cause proliferation [46], and enhance the interferon-γ mediated expression of class II antigens [8], a phenomenon of considerable potential relevance given the proposed role of neuroglial class II antigens in limiting HSV infection in the central nervous system [30]. In the periphery, TNF has been shown to enhance neurotransmitter release from motorneuron endplates [10], and to affect both calcium currents [49] and nicotinic responses [51] in cultured sympathetic neurons. In addition, TNF has been shown in cultured Schwann cells to inhibit proliferation, to decrease gap junction conduction [11], and to down-regulate myelin-associated glycoprotein expression, thereby inhibiting neurite outgrowth [45]. However, we found no evidence for expression of TNFR mRNA over Schwann cells in either normal or infected trigeminal ganglia, suggesting that TNFR mRNA is expressed below the level of detection with radiolabeled riboprobes, or, alternatively, that those TNF-mediated Schwann cell responses observed in vitro are either indirect, or related to cell growth in culture.

Autoradiographic signal for both TNFR subtypes was observed over the arachnoid layers surrounding the trigeminal ganglia. This is an important finding given the well documented increase in TNF in the central nervous system in the setting of meningitis [2,4,22,31,35–38,40,60], where cerebral spinal fluid levels of TNF have been inversely correlated with level of consciousness [2,4,35,36], and local TNF has been shown to elicit many of the injurious cerebral metabolic and circulatory changes that accompany this condition [35,44,58]. Recently, Sanna and associates have shown that intracerebroventricular administration of the bacterial lipopolysaccharide, a component of the outer membrane of Gram-negative bacteria, mimics the rise in central TNF seen with bacterial meningitis [43].

Acute ocular HSV infection was accompanied by an intense leukocytic infiltrate into the medial, or ophthalmic, portion of the trigeminal ganglion. Our in situ hybridization results demonstrated that these infiltrating white blood cells expressed high levels of both p55 and p75 TNFR mRNA. While a slight increase in or delayed expression of endogenous TNFR mRNA cannot be ruled out with these studies, the results strongly suggest that the increase in TNFR mRNA in the trigeminal ganglia observed in the setting of acute ocular HSV infection is due to an influx of receptor-bearing white blood cells. Similar findings have

been reported for TNFR mRNA in mouse brain following infection with LCMV [53], and in eye after acute infection with McKrae strain HSV [15].

Acknowledgements

This work was supported by a Career Development Award from Research to Prevent Blindness (T.P.M.) and NIH Grants EY10008 and EY02162 (T.P.M.), MH47680 (F.E.B.), and MH50426 (I.L.C.).

References

[1] Ada, G. and Blanden, R.V., CTL immunity and cytokine regulation in viral infection, *Res. Immunol.*, 145 (1994) 625–628.

[2] Akalin, H., Akdis, A.C., Mistik, R., Helvaci, S. and Kilicturgay, K., Cerebrospinal fluid interleukin-1β/interleukin-1 receptor antagonist balance and tumor necrosis factor-a concentrations in tuberculous, viral and acute bacterial meningitis. *Scand. J. Infect. Dis.*, 26 (1994) 667–674.

[3] Aranguez, I., Torres, C. and Rubio, N., The receptor for tumor necrosis factor on murine astrocytes: characterization, intracellular degradation, and regulation by cytokines and Theiler's murine encephalomyelitis virus, *Glia*, 13 (1995) 185–194.

[4] Arditi, M., Manogue, K.R., Caplan, M. and Yogev, R., Cerebrospinal fluid cachetin/tumor necrosis factor-α and platelet-activating factor concentrations and severity of bacterial meningitis in children, *J. Infect. Dis.*, 162 (1990) 139–147.

[5] Baringer, J.R. and Griffith, J.F., Experimental herpes simplex encephalitis: Early neuropathologic changes, *J. Neuropathol. Exp. Neurol.*, 29 (1970) 89–104.

[6] Barna, B.P., Barnett, G.H., Jacobs, B.S. and Estes, M.L., Divergent responses of human astrocytoma and non-neoplastic astrocytes to tumor necrosis factor alpha involve the 55 kDa tumor necrosis factor receptor, *J. Neuroimmunol.*, 43 (1993) 185–190.

[7] Barnum, Sc.R., Jones, J.L. and Benveniste, E.N., Interleukin-1 and tumor necrosis factor-mediated regulation of C3 gene expression in human astroglioma cells, *Glia*, 7 (1993) 225–236.

[8] Benveniste, E.N., Sparacio, S.M. and Bethea, J.R., Tumor necrosis factor-alpha enhances interferon-gamma-mediated class II antigen expression on astrocytes, *J. Neuroimmunol.*, 25 (1989) 209–219.

[9] Beutler, B., TNF, immunity and inflammatory disease: Lessons of the past decade, *J. Invest. Med.*, 43 (1995) 227–235.

[10] Caratsch, D.G., Santoni, A. and Eusebi, F., Interferon-α, β and tumor necrosis factor enhance the frequency of miniature end plate potentials at rat neuromuscular junction, *Neurosci. Lett.*, 166 (1994) 97–100.

[11] Chandross, K.J., Chanson, M., Spray, D.C. and Kessler, J.A., Schwann cell proliferation and intercellular coupling are coordinately regulated, *Soc. Neurosci. Abstr.* 19 (1993) 1722.

[12] Chao, C.C. and Hu, S., Tumor necrosis factor-α potentiates glutamate neurotoxicity in human fetal brain cell cultures, *Dev. Neurosci.*, 16 (1994) 172–179.

[13] Cheng, B., Christakos, S. and Mattson, M.P., Tumor necrosis factors protect neurons against metabolic-excitotoxic insults and promote maintenance of calcium homeostasis, *Neuron*, 12 (1994) 139–153.

[14] Cunningham, E.T., Jr. and De Souza, E.B., Localization of type I IL-1 receptor mRNA in brain and endocrine tissues using in situ hybridization histochemistry. In E.B. De Souza (Ed.), *Methods in Neuroscience. Neurobiology of Cytokines. Part A*, CRC Press, New York, 1994, Chapter 7, pp. 112–127.

[15] Cunningham, E.T., Jr., Stalder, A., Sanna, P.P., Liu, S.S., Bloom, F.E., Howes, E.L., Jr., Campbell, I.L. and Margolis, T.P., Localization of tumor necrosis factor receptor messenger RNA in normal and herpes simplex virus infected mouse eyes, *Invest. Ophthalmol. Vis. Sci.*, 38 (1996) 9–15.

[16] Elenkow, I.J., Kovacs, K., Duda, E., Stark, E. and Vizi, E.S., Presynaptic inhibitory effect of TNF-a on the release of noradrenaline in isolated median eminence, *J. Neuroimmun.*, 41 (1992) 117–120.

[17] Feduchi, E., Alonso, M.A. and Carrasco, L., Human gamma interferon and tumor necrosis factor exert a synergistic blockage on the replication of herpes simplex virus, *J. Virol.*, 63 (1989) 398–409.

[18] Fraser, N.W. and Valyi-Nagy, T. Viral, neuronal and immune factors which may influence herpes simplex virus (HSV) latency and reactivation, *Microbial Pathogenesis*, 15 (1993) 83–91.

[19] Geist, L.J. and Hunninghake, G.W., Cytokine gene regulation by viral gene products, *J. Lab. Clin. Med.*, 126 (1995) 517–520.

[20] Gelbard, H.A., Dzenko, K.A., DiLoreto, D., del Cerro, C., del Cerro, M. and Epstein, L.G., Neurotoxic effects of tumor necrosis factor alpha in primary human neuronal cultures are mediated by activation of the glutamate AMPA receptor subtype: Implications for AIDS neuropathogenesis, *Dev. Neurosci.*, 15 (1993) 417–422.

[21] Ghiasi, H., Wechsler, S.L., Kaiwar, R., Nesburn, A.B. and Hofman, F.M., Local expression of tumor necrosis factor alpha and interleukin-2 correlates with protection against corneal scarring after ocular challenge of vaccinated mice with herpes simplex virus type 1, *J. Virol.*, 69 (1995) 334–340.

[22] Glimaker, M., Kragsbjerg, P., Forsgren, M. and Olcen, P., Tumor necrosis factor-alpha (TNF-alpha) in cerebrospinal fluid from patients with meningitis of different etiologies: high levels of TNF-alpha indicate bacterial meningitis, *J. Infect. Dis.*, 167 (1993) 882–889.

[23] Goodpasture, E. and Teague, O., Transmission of the virus of herpes fibrilis along nerves in experimentally infected rabbits. *J. Med. Res.*, 44 (1923) 139–184.

[24] Goodwin, R.G., Anderson, D., Jerzy, R., Davis, T., Brannan, C.I., Copeland, N.G., Jenkins, N.A. and Smith, C.A., Molecular cloning and expression of the type 1 and type 2 murine receptors for tumor necrosis factor, *Mol. Cell Biol.*, 11 (1991) 3020–3026.

[25] Grassi, F., Mileo, A.M., Monaco, L., Punturieri, A., Santoni, A. and Eusebi, F., TNF-α increases the frequency of spontaneous miniature synaptic currents in cultured rat hippocampal neurons, *Brain Res.*, 659 (1994) 226–230.

[26] Ignatowski, T.A. and Spengler, R.N., Tumor necrosis factor-α: Presynaptic sensitivity is modified after antidepressant drug administration, *Brain Res.*, 665 (1994) 293–299.

[27] Itoyama, Y., Sekizawa, T., Openshaw, H., Kogure, K. and Kuroiwa, Y., Immunocytochemical localization of herpes simplex antigen in the trigeminal ganglia of mice, *J. Neurol. Sci.*, 66 (1984) 673–681.

[28] Kinouchi, K., Brown, G., Pasternak, G. and Donner, D.B., Identification and characterization of receptors for tumor necrosis factor-α in the brain, *Biochem. Biophy. Res. Comm.*, 181 (1991) 1532–1538.

[29] Knotts, F., Cook, M. and Stevens, J., Pathogenesis of herpetic encephalitis in mice after ophthalmic inoculation, *J. Infect. Dis.*, 130 (1974) 16–27.

[30] Lewandowski, G.A., Lo, D. and Bloom, F.E., Interference with major histocompatibility complex class II-restricted antigen presentation in the brain by herpes simplex virus type 1: A possible mechanism of evasion of the immune response, *Proc. Natl. Acad. Sci. USA*, 90 (1993) 2005–2009.

[31] Lopez-Cortes, L.F., Cruz-Ruiz, M., Gomez-Mateos, J., Jimenez-Hernandez, D., Palomino, J. and Jimenez, E., Adenosine deaminase activity in the CSF of patients with aseptic meningitis: utility in the diagnosis of tuberculous meningitis or neurobrucellosis, *Clin. Infect. Dis.*, 16 (1993) 534–539.

[32] Louis, J.C., Magal, E., Takayama, S. and Varon, S., CNTF protection of oligodendrocytes against natural and tumor necrosis factor-induced death, *Science*, 259 (1993) 689–692.

[33] Margolis, T.P., Sedarati, F., Dobson, A.T., Feldman, L.T. and

Stevens, J.G., Pathways of viral gene expression during acute neuronal infection with HSV-1, *Virology*, 189 (1992) 150–160.

[34] Margolis, T.P., Togni, B., La Vail, J.H. and Dawson, C., Identifying HSV infected neurons after ocular inoculation, *Curr. Eye Res.*, 6 (1987) 119–126.

[35] Mustafa, M.M., Lebel, M.H., Ramilo, O., Olsen, K.O., Reisch, J.S., Beutler, B. and McCraken, G.H., Jr., Correlation of interleukin-1β and cachectin concentrations in cerebrospinal fluid and outcome from bacterial meningitis, *J. Pediatr.*, 115 (1989) 208–213.

[36] Mustafa, M.M., Ramilo, O., Saez-Llorens, X., Mertsola, J., Magnes, R.R. and McCracken, G.H., Jr., Prostaglandin E2 and I2, interleukin-1b and tumor necrosis factor in cerebrospinal fluid of infants and children with bacterial meningitis, *Pediatr. Infect. Dis. J.*, 8 (1989) 921–922.

[37] Nadal, D., Leppert, D., Frei, K., Gallo, P., Lamche, H. and Fontana, A., Tumor necrosis factor-α in infectious meningitis, *Arch. Dis. Child.*, 64 (1989) 1274–1279.

[38] Ohga, S., Aoki, T., Okada, K., Akeda, H., Fujioka, K., Ohshima, A., Mori, T., Minamishima, I. and Ueda, K., Cerebrospinal fluid concentrations of interleukin-1 beta, tumour necrosis factor-alpha, and interferon gamma in bacterial meningitis, *Arch. Dis. Child.*, 70 (1994) 123–125.

[39] Poli, G. and Fauci, A.S., Cytokine modulation of HIV expression, *Semin. Immunol.*, 5 (1993) 165–173.

[40] Ramilo, O., Mustafa, M.M., Porter, J., Saez-Llorens, X., Mertsola, J., Olsen, K.D., Luby, J.P., Beutler, B. and McCracken, G.H., Jr., Detection of interkeukin-1β but not tumor necrosis factor-a in cerebrospinal fluid of children with aseptic meningitis, *AJDC*, 144 (1990) 349–352.

[41] Robbins, D.S., Shirazi, Y., Drysdale, B.E., Lieberman, A., Shin, H.S. and Shin, M.L., Production of cytotoxic factor for oligodendrocytes by stimulated astrocytes, *J. Immunol.*, 139 (1987) 2593–2597.

[42] Rossol-Voth, R., Rossol, S., Schutt, K.H., Corridori, S., de Cian, W. and Falke, D., In vivo protective effect of tumour necrosis factor a against experimental infection with herpes simplex virus type 1, *J. Gen. Virol.*, 72 (1991) 143–147.

[43] Sanna, P.P., Weiss, F., Samson, M.E., Bloom, F.E. and Pich, E.M., Rapid induction of tumor necrosis factor a in cerebrospinal fluid after intracerebroventricular injection of lipopolysaccharide revealed by a sensitive capture immuno-PCR assay, *Proc. Natl. Acad. Sci. USA*, 92 (1995) 272–275.

[44] Saukkonen, K., Sande, S., Cioffe, C., Wolpe, S., Sherry, B., Cerami, A. and Tuomanen, E., The role of cytokines in the generation of inflammation and tissue damage in experimental gram-positive meningitis, *J. Exp. Med.*, 171 (1990) 439–448.

[45] Schneider-Schaulies, J., Kirchoff, F., Archelos, J. and Schachner, M., Down-regulation of myelin-associated glycoprotein on Schwann cells by interferon-γ and tumor necrosis factor-α affects neurite outgrowth, *Neuron*, 7 (1991) 995–1005.

[46] Selmaj, K.M., Faroq, M., Norton, W.T., Raine, C.S. and Brosnan, C.F., Proliferation of astrocytes in vitro in response to cytokines: A primary role for tumor necrosis factor, *J. Immunol.*, 144 (1990) 129–135.

[47] Shun-Hua, C., Oakes, J.E. and Lausch, R.N., Synergistic anti-herpes effect of TNF-α and IFN-γ in human corneal epithelial cells with that in corneal fibroblasts, *Antiviral Res.*, 25 (1994) 201–213.

[48] Simmons, A., Tscharke, D. and Speck, P., The role of immune mechanisms in control of herpes simplex virus infection of the peripheral nervous system, *Curr. Top. Microbiol. Immunol.*, 179 (1992) 31–56.

[49] Soliven, B. and Albert, J., Tumor necrosis factor modulates Ca^{2+} currents in cultured sympathetic neurons, *J. Neurosci.*, 12 (1992) 2665–2671.

[50] Soliven, B., Szuchet, S. and Nelson, D.J., Tumor necrosis factor inhibits K+ current expression in cultured oligodendrocytes, *J. Membr. Biol.*, 124 (1991) 127–137.

[51] Soliven, B. and Wang, N., Tumor necrosis factor-α regulates nicotinic responses in mixed culture of sympathetic neurons and non-neuronal cells, *J. Neurochem.*, 64 (1995) 883–894.

[52] Speck, P.G. and Simmons, A., Divergent molecular pathways of productive and latent infection with a virulent strain of herpes simplex virus type 1, *J. Virol.*, 65 (1991) 4001–4005.

[53] Stalder, A. and Campbell, I.L., Cytokine receptor gene expression in lymphocytic choriomeningitis, *Soc. Neurosci. Abstr.*, 326 (1993).

[54] Stalder, A. and Campbell, I.L., Simultaneous detection of multiple cytokine receptor mRNAs by RNase protection assay in LPS-induced endotoxemia, *Lymphokine and Cytokine Res.*, 13 (1994) 107–109.

[55] Talley, A.K., Dewhurst, S., Perry, S.W., Dollard, S.C., Gummuluru, S., Fine, S.M., New, D., Epstein, L.G., Gendelman, H.E. and Gelbard, H.A., Tumor necrosis factor α-induced apoptosis in human neuronal cells: protection by the antioxidant *N*-acetylcysteine and genes *bcl-2* and *crmA*, *Mol. Cell Biol.*, 15 (1995) 2359–2366.

[56] Tancredi, V., D'Arcangelo, G., Grassi, F., Tarroni, P., Palmieri, G., Santoni, A. and Eusebi, F., Tumor necrosis factor alters synaptic transmission in rat hippocampal slices, *Neurosci. Lett.*, 146 (1992) 176–178.

[57] Tracey, K. and Cerami, A., Tumor necrosis factor: a pleiotropic cytokine and therapeutic target, *Annu. Rev. Med.*, 45 (1994) 491–503.

[58] Tureen, J., Effect of recombinant human tumor necrosis factor-alpha on cerebral oxygen uptake, cerebrospinal fluid lactate, and cerebral blood flow in the rabbit: Role of nitric oxide, *J. Clin. Invest.*, 95 (1995) 1086–1091.

[59] Waled, I., Podlech, J. and Falke, D., Enhancement by TNF-α of reactivation and replication of latent herpes simplex virus from trigeminal ganglia of mice, *Arch. Virol.*, 140 (1995) 987–992.

[60] Waage, A., Halstensen, A., Shalaby, R., Brandtz, P., Kierule, P. and Espevik T., Local production of tumor necrosis factor α, interleukin 1, and interleukin 6 in meningococcal meningitis, *J. Exp. Med.*, 170 (1989) 1859–1867.